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What is claimed is:

5 1. A recombinant DNA construct for expressing
at least one heterologous protein in the plastids of
higher plants, said construct comprising a 5' regulatory
region which includes a promoter element, a leader
sequence and a downstream box element operably linked to
10 a coding region of said at least one heterologous
protein, said chimeric regulatory region enhancing
translational efficiency of an mRNA molecule encoded by
said DNA construct.

15 2. A vector comprising the DNA construct of
claim 1.

20 3. A recombinant DNA construct as claimed in
claim 1, said 5' regulatory region being selected from
the group consisting of PrnnLatpB+DBwt, SEQ ID NO:1,
PrnnLatpB-DB, SEQ ID NO:2, PrnnLatpB+DBm, SEQ ID NO:3,
PrnnLclpP+DBwt, SEQ ID NO: 4, PrnnlcpP-DB, SEQ ID NO:5,
PrnnLrbcL+DBwt, SEQ ID NO:6, PrnnLrbcL-DB, SEQ ID NO:7,
PrnnLrbcL+DBm, SEQ ID NO:8, PrnnLpsbB+DBwt, SEQ ID NO:9,
25 PrnnLpsbB-DB, SEQ ID NO:10, PrnnLpsbA+DBwt, SEQ ID NO:
11, PrnnLpsbA-DB, SEQ ID NO:12, PrnnLpsbA-DB(+GC), SEQ
ID NO:13.

30 4. A recombinant DNA construct as claimed in
claim 1, said 5' regulatory region being selected from
the group consisting of PrnnLT7g10+DB/Ec, SEQ ID NO:14,

*Sub
A1* >

Sub
At
2000/07/10

PrnLT7g10+DB/pt, SEQ ID NO:15, PrnLT7g10-DB, SEQ ID NO:15.

5. A vector comprising a DNA construct as
5 claimed in claim 1.

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6. A DNA construct as claimed in claim 1,
said downstream box element having a sequence selected
from the group consisting of
10 5'TCCAGTCACTAGCCCTGCCTTCGGCA'3 and
5'CCCAGTCATGAATCACAAAGTGGTAA'3.

7. A DNA construct as claimed in claim 1,
wherein said heterologous protein is expressed from a
15 bar gene encoded by *S. hydroscopicus* said bar gene
inserted into a plasmid selected from the group
consisting of pK012, and pJEK3, said pJEK3 having the
sequence of SEQ ID NO: 18.

8. A DNA construct as claimed in claim 1,
wherein said heterologous protein is expressed from a
synthetic bar encoding nucleic acid, said synthetic bar
nucleic acid having selected from the group consisting
of SEQ ID NO: 19 and SEQ ID NO:20.

9. A DNA construct as claimed in claim 1,
said at least one heterologous protein comprising a
fusion protein.

10. A DNA construct as claimed in claim 9,
said fusion protein having a first and second coding
region operably linked to said 5' regulatory region such
that production of said fusion protein is regulated by

said 5' regulatory region, said first coding region encoding a selectable marker gene and said second coding region encoding a fluorescent molecule to facilitate visualization of transformed plant cells.

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11. A vector comprising the DNA construct of claim 10.

10 12. A DNA construct as claimed in claim 9, said fusion protein consisting of an aadA coding region operably linked to a green fluorescent protein coding region.

15 ~~cus pin~~ 13. A DNA construct as claimed in claim 10, said aadA coding region being operably linked to said green fluorescent protein coding region via a nucleic acid molecule encoding a peptide linker having a sequence selected from the group consisting of ELVEGKLELVEGLKVA and ELAVEGKLEVA.

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14. A DNA construct as claimed in claim 10, said construct having a sequence selected from the group of SEQ ID NOS: 21-25 and 27.

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15. A plasmid for transforming the plastids of higher plants, said plasmid being selected from the group consisting of pHK30(B), pHK31(B), pHK60, pHK32(B), pHK33(B), pHK34(A), pHK35(A), pHK64(A), pHK36(A), pHK37(A), pHK38(A), pHK39(A), pHK40(A),
30 pHK41(A), pHK42(A), pHK43(A), pMSK56, pMSK57, pMSK48, pMSK49, pMSK35, pMSK53 and pMSK54.

16. A transgenic plant containing a plasmid as claimed in claim 15.

17. A transgenic plant as claimed in claim 15, said plant being selected from the group consisting of monocots and dicots.

18. A method for producing transplastomic monocots, comprising:

- 10 a) obtaining embryogenic cells;
- b) exposing said cells to a heterologous DNA molecule under conditions whereby said DNA enters the plastids of said cells, said heterologous DNA molecule encoding at least one exogenous protein, said at least
- 15 one exogenous protein encoding a selectable marker;
- c) applying a selection agent to said cells to facilitate sorting of untransformed plastids from transformed plastids, said cells containing transformed plastids surviving and dividing in the presence of said
- 20 selection agent;
- d) transferring said surviving cells to selective media to promote shoot regeneration and growth; and
- e) rooting said shoots, thereby producing
- 25 transplastomic monocot plants.

19. A method as claimed in claim 18, wherein said heterologous DNA molecule is introduced into said plant cell via a process selected from the group consisting of

30 biolistic bombardment, Agrobacterium-mediated transformation, microinjection and electroporation.

20. A method as claimed in claim 18, wherein protoplasts are obtained from said embryogenic cells and said heterologous DNA molecule is delivered to said protoplasts by exposure to polyethylene glycol.

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21. A method as claimed in claim 18, wherein said selection agent is selected from the group consisting of streptomycin, and paromomycin

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22. A monocot transformed via the method of claim 18.

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23. A transformed monocot plant as claimed in claim 22, said monocot plant being selected from the group consisting of maize, millet, sorghum, sugar cane, rice, wheat, barley, oat, rye, and turf grass.

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24. A method for producing transplastomic rice plants, said method comprising:

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a) obtaining embryogenic calli;
b) inducing proliferation of calli on modified CIM medium;

c) obtaining embryogenic cell suspensions of said proliferating calli in liquid AA medium;

d) bombarding said embryogenic cells with microprojectiles coated with plasmid DNA;

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e) transferring said bombarded cells to selective liquid AA medium;

f) transferring said cells surviving in AA medium to selective RRM regeneration medium for a time period sufficient for green shoots to appear; and

g) rooting said shoots in a selective MS salt medium.

25. A method as claimed in claim 24, said plasmid DNA being selected from the group of plasmids consisting of pMSK35 and pMSK53, pMSK54 and pMSK49.

26. A transplastomic rice plant produced by the method of claim 24.

27. A method for containing transgenes in transformed plants, comprising:

a) determining the codon usage in said plant to be transformed and in microbes found in association with said plant; and

b) genetically engineering said transgene sequence via the introduction of rare codons to abrogate expression of said transgene in said plant associated microbe.

28. A method as claimed in claim 27, wherein said transgene is a bar gene and said rare codons are arginine encoding codons selected from the group consisting of AGA and AGG.

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